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A BACTERIOLOGIC STUDY OF SARDINES *

MAUD MASON OBST

The yearly loss from the swelling of cans of sardines has been stated by the canners to be 1% or higher, and in some cases it has been reported to be 30% of the output. Prescott and Underwood,[1] in the study of the canning of clams and lobsters, found several species of living bacteria in the swelled goods. Cathcart[2] isolated 4 organisms from canned sardines. These included B. coli, 2 organisms which were pathogenic to guinea-pigs when injected intraperitoneally and 1 nonpathogenic. In 1913, during a preliminary investigation of the canning of clams in an unpublished account, Dr. Payn B. Parsons[3] reported the finding of gas-producing facultative anaerobic bacteria in the commercially processed goods. He found it necessary to process the clams at 240 F. in order to insure complete sterilization. This fact, of course, suggested the presence of bacterial spores, but they were not demonstrated culturally, nor was their relation to oxygen definitely determined. It was, therefore, considered probable that such organisms might be the cause of the swelled cans of sardines. It seemed possible also that the organisms which caused the trouble in clams might be identical.

A number of swelled cans of sardines received from packers at various places along the coast of Maine, including Eastport, Lubec, North Lubec, Machiasport, Jonesport, Boothbay Harbor, and Rockland were examined for both aerobic and anaerobic bacteria. The cans were carefully sterilized on the outside by flaming with alcohol and opened with a sterilized can opener. In many cases when the cans appeared badly swollen their contents were entirely decomposed, all connective tissue and even the bone tissue of the sardines having been destroyed until no resemblance of a fish form remained. The contents were reduced to a soft pasty mass full of gas bubbles and this mass emitted a disagreeable odor of sourness and hydrogen sulphid.

Aerobic plate cultures were made from several cans on fish, dextrose, and nutrient agar and in dextrose and nutrient broth. In practically no instance were any living aerobic bacteria found, except in those cans which had been burst by swelling. From each of the 287 cans examined, which were not burst,

Received for publication Oct. 15, 1918.

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* This article deals with the bacteriologic results which were obtained during the studies conducted in the sardine industry in collaboration with the Animal Physiological Laboratory of the Bureau of Chemistry, Washington.

[1] Technology Quarterly, 1897, 10, p. 183.

[2] Jour. Hyg., 1906, 6, p. 248.

[3] Bacteriologist, Bureau of Chemistry, Washington, D. C.

an anaerobic organism was found in pure culture. This organism was obtained only in its spore stage. Morphologically and in many of its cultural characteristics it closely resembles the bacillus of symptomatic anthrax (B. rauschbrand). It, however, is probably more nearly related to the organism described by Ivar Nielsen[4] as Bacillus walfischrauschbrand and will thus be designated hereinafter. Its interesting characteristics are its ability to form gas from protein or carbohydrate mediums, and the rapidity with which it forms spores. When grown under laboratory conditions, no vegetative cells without spores were found in 8-hour old cultures. It is hoped that the method of cell division of this organism may be studied further. Only 10 cans of imported (Norwegian) sardines have been examined, and of these, 7 which were appreciably swollen, contained B. walfischrauschbrand.

In cannery practice the sealed cans of sardines are heated in an open bath of boiling water. The time of processing varies in different factories from 1¼-2½ hours. The water is usually heated either by free steam entering at the bottom or by steam coils. The tanks in which the following experiments were conducted were about 4 feet long, 2½ feet wide, and 4 feet deep, and held about 65 cases of cans. Cans of sardines were inoculated with B. walfischrauschbrand and placed in various parts of the boiling tanks. Of 11 cans placed near the surface 7 were processed 1½ hours and the remainder for 2½ hours. The B. walfischrauschbrand spores were recovered from 5 of the 7 cans while cultures from only 1 of the 4 which were heated for 2½ hours showed growth. Of the other cans, only 1 showed living organisms after processing.

Temperatures were taken of the contents of cans placed well beneath the surface of the water in the boiling tanks by means of a thermocouple attached to a potentiometer. It was found that in one case 12 minutes were required to heat the interior of the can from 45-99 C. In another attempt, 25 minutes' boiling raised the temperature from 36-100 C. If 25 minutes are taken from 75 minutes, the time of actually heating cans in some factories, it does not allow a very great margin over the time absolutely necessary for killing the spores of the bacteria. In many instances, too, cans are allowed to float in the water or remain only partially covered with water. This prevents thorough processing. Cans, however, which are completely filled without leaving any air space and which do not leak, do not float. Their own weight holds them beneath the water.

An experiment was made to determine if bacteria lived through the process of frying in an oil bath at a temperature of 240 F. It was found that B. walfischrauschbrand planted in the meaty portion of a herring[5] which was protected by some portion of other herring both above and below was not killed. In one instance a stomach containing feed was found in a little heap of three fish which overlapped one another, and from it was isolated a bacillus which is described later in this paper as Bacillus B.

In an attempt to learn from what source B. walfischrauschbrand found its way into the cans, moist, greasy dirt was scraped from the inside of the pickling tanks of the sardine canneries and cultures prepared. The spores of this organism were present in large numbers. They were also obtained from dirt on the floors beneath the flaking machines in certain factories. Since it has been impossible to demonstrate the presence of the vegetative form of the bacillus even in the laboratory mediums, it could not be definitely determined whether multiplication takes place under the conditions where it has been found or whether it is present in the spore form only. While searching for B. walfischrauschbrand in the factory, it was found that B. welchii, also a spore-forming, gas-producing bacillus, entered 3 of the factories through the water supply used for washing and pickling the herring. The presence of B. welchii indicates sewage, although it gives no clue to the recentness of the deposit. In one instance its source was located in the mud directly beneath the opening of the intake water pipe. The end of this pipe was about 2 feet above the mud at the time of sampling. The shore was sloping at this point and the mud showed evidences of having been recently dug up by the prow of a boat. Any boat coming into the region at low water disturbed the water and caused that which entered the factory to show a large amount of sediment. In another instance the intake pipe to the water supply for washing and pickling the fish had its opening directly on the gravel bottom of the shore about 15 feet from the outlet of one of the main sewers of the town. It was thus clear that dirt or filth in the factories might be one source of pollution in the product.

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[4] Centralbl. f. Bakteriol., 1880, 7, p. 267; Kitt: Th. Bacterienkunde, 1903, p. 301.

[5] "Herring" is used throughout this paper to designate the sea herring or Clupea harengus which is used for sardines along the Maine coast.

These organisms were next sought in and on the fish as they came to the factories. In November, 1915, herring were taken alive from a weir near Boothbay Harbor, Maine, in sterile containers. Sterilized glass fruit jars, which were kept sterile on the outside by being tied in cotton bags before being autoclaved were used for containers. After a little practice the live herring were readily dipped with the sterile jars directly out of the seine before they were lifted from the water. At the laboratory the herring were dissected with sterile instruments, and sections placed in sterile culture tubes. Dextrose agar was then poured into the tubes and allowed to solidify. This was found to provide an anaerobic condition which permitted the growth of strict and facultative anaerobes. Tube and aerobic plate cultures were made at first with both dextrose and fish agar, but as there appeared to be little advantage in using the fish agar for tube cultures it was omitted.

The flesh of the herring fresh from the water was aseptically removed by holding the fish with sterile forceps while the skin was cut away from the thick meat on the side of the fish, and then a portion of this meat was cut out with sterile implements. To between 2.5 and 5 gm. of the flesh thus obtained was added sterile water in the ratio of 1:9. This was then shaken with sterile shot and 1 c c portions were plated on dextrose agar and fish agar. The remainder in the flask was then covered with fish agar. All were incubated at 37 C. Several fish thus examined showed the growth of a few organisms, but only 2 of 56 showed more than 100 bacteria to the gm. The majority of the plates showed from 1-5 colonies of bacteria on the 1 c c plates of the above liquid. These may have been accidental contaminations. In no instance were gas-producing bacteria present.

Sections of the stomach and intestines were aseptically removed, split lengthwise, washed with sterile water, and cultures made of the entire portions, the wash water and sections separately. In several instances special tests were made to determine the sterility of the stomach and intestines in the absence of feed. Of 94 herring thus examined in which the absence of visible feed was noted at the time of dissection, 13 only showed the presence of any bacteria in the stomach or intestines. In many instances the stomachs were free from both feed and bacteria, when partially digested feed, living bacteria, and occasionally gas were present in the intestines. The gas was usually found only after the herring had been held from 5-12 hours after removal from the water. In those fish in which the stomachs contained feed the dorsal ends were often markedly distended with gas. B. walfischrauschbrand was found in three instances. No other bacteria were isolated. Three herring which contained feed in the form of tiny fish were examined. These contained no bacteria in their stomachs. In nearly all fish which contained the feed known among the fishermen as "red feed," bacteria capable of producing gas were found. Bacillus B. was found either in pure culture or accompanied by an aerobic white micrococcus in the digestive organs of many herring which contained copepods. Where the schizopod eyes were present in the stomachs of herring, B. walfischrauschbrand was frequently found, in several instances alone, yet often accompanied by Bacillus B. From this work it is suggested that the very simple digestive tract of the herring mechanically frees itself of bacteria shortly after all feed is eliminated (Plate I).

The gills of the herring were almost always found to harbor living bacteria whenever they were present in any part of the fish and in about 40 herring of which all other portions were sterile. Bacillus B. was isolated in only one instance from the gills, but B. walfischrauschbrand was found in all others which showed gas-producing organisms. It seems probable that the gills may filter the spores of the latter bacillus from the water which passes through and hold them in the spore stage. The herring from which B. walfischrauschbrand was isolated were obtained from the following places:

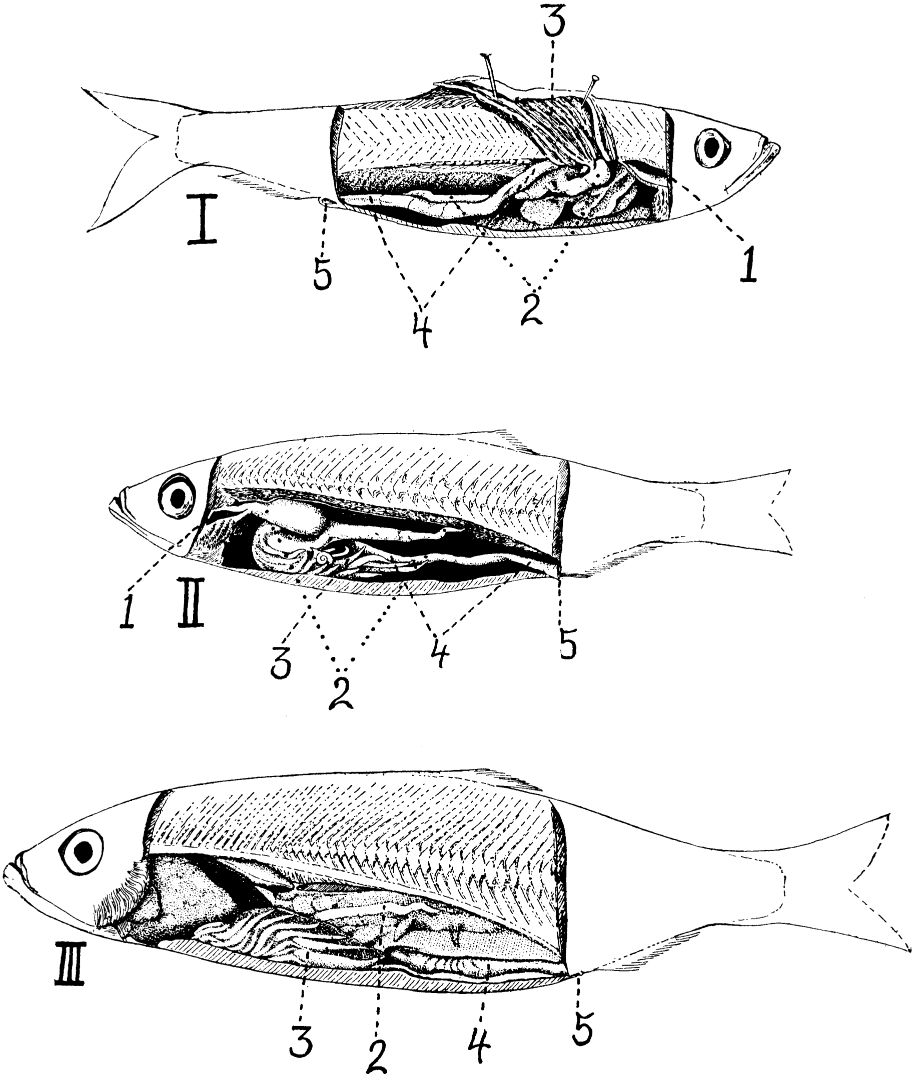
1. Weir near Boothbay Harbor, Maine.
2. Weir at Hog Island, South Bay, near North Lubec, Maine.
3. Seine at Denbrow Pt., Cobscock Bay, near North Lubec, Maine.
4. Weir at L'Etang Harbor, near L'Etang Peninsula, N. B. Canada.
5. Boat at Machiasport Wharf, Machiasport, Maine.

Bacillus B. was found in fish from the third and fourth places mentioned, and also in fish obtained from boats in the harbor at Eastport, Maine, probably brought from Irish Channel, near Deer Island, Canada, and from Back Bay, Canada. It was obtained in herring taken direct from a weir near Three Islands off the southeast coast of Grand Manan Island, Canada.

From several herring the stomach contents, comprised apparently entirely of copepods, were removed and incubated in a tube with no added mediums under anaerobic conditions. Gas accompanied by a foul odor was present after 5 hours' incubation at 37 C. Bacillus B. was isolated. It may be noted that under the description of Bacillus B. it was found to produce gas rapidly in the presence of horse blood in sugar mediums.

From this work it seemed evident that B. walfischrauschbrand and Bacillus B. might accompany the feed of the herring, especially the schizopods and copepods while living, and on this feed be carried into the digestive tract and thus be brought to the factory. These forms of feed were therefore collected fresh from the water and examinations made.

The schizopods were found in large numbers in the water around Wilson's Beach, Campobello Island, in the cove on Moose Island above Dog Island; around the wharves at Eastport and Lubec, and in smaller numbers in the southern part of South Bay. These waters with the exception of South Bay all contain waste products discarded from fresh fish trimmings or from various factories, and the portion of South Bay where the schizopods were found is rich in organic matter and does not receive much purification by the tide. The food supply probably partially influences the location of the schizopods and, doubtless, their bacterial associates. It seemed significant that in regions where



1. Mouth.
2. Stomach.
3. Appendices to the dorsal end of the stomach.
4. Intestine.
5. Anus.

the sardine packers and others avoided polluting the water with waste products schizopods were seldom seen. The schizopods were most easily caught at about 3 hours after high water when they were found in masses among the rocks which were covered with sea-weed. They were scooped with a net fastened into the hoop of a dip-net handle. The net used was made of a coarse mesh linen scrim sewed to a 6 inch wide band of heavy cotton cloth. The lower part of the net was left open and a gathering string sewed in so that it could be tied firmly around the neck of a glass jar. For this investigation sterile jars of the Lightning type were used and the net was autoclaved in a cotton bag in which it was kept until used. The schizopods were caught in sterile containers and kept alive in sea-water until they reached the laboratory. It was noted that when they were placed in 30% alcohol at room temperature for 24 hours the schizopods emitted a large volume of gas and disagreeable odor. The surface of the alcoholic solution was often covered with foam. The crawfish, which has a shell of calcareous chitin, will close this shell with its muscles and hold out preservatives until long after death, thus allowing the decay of the meat within the shell by bacterial action. This evidently happens in the case of the schizopods, which have a similar chitinous shell.

Anaerobic and aerobic cultures were made of the entire schizopods and of sections cut aseptically from the bodies. Not all schizopods showed bacterial growth. Many of the whole bodies and sections of the shell covering showed aerobic bacteria but they were not identified. Sections from the thoracic and digestive regions showed the presence of bacteria including B. walfischrauschbrand, and in two instances Bacillus B. The dorsal sections of the body showed no growth.

In the complete report on this subject, Dr. Weber calls attention to the care exercised by the French sardine industry in securing clean bait for catching the fish. The following is quoted from his report: "So particular are the fishermen and the manufacturers to avoid the decomposition when fish are feedy, that the strictest attention is given to the quality of the bait. In early times in this industry, the use of a prepared bait containing especially powdered prawn and shrimp was forbidden by royal decree. As late as 1853, the use of this bait was forbidden. It was held that it spoiled the fish by facilitating decomposition." If the shrimp used contained originally B. walfischrauschbrand, the spores of this bacillus could have resisted the process of drying and powdering by which the bait was prepared. It would then have been capable of facilitating the decomposition of the fish.

The copepods were collected in large quantities off Whale Cove between Swallow Tail Light and Bishops Rock, Grand Manan Island, Canada, and in small quantities directly east from Grand Manan, by using a copepod net,[5] sterilized, with a sterile glass jar attached.

Individual copepods were cultured in dextrose and fish agar, and masses of the copepods were placed in large tubes covered with the above mediums, and incubated under anaerobic conditions. Abundant gas formed in the latter after 6 hours' incubation at 37 C. and Bacillus B. was isolated. Nearly all of the individual copepods which were incubated showed the presence of Bacillus B. B. walfischrauschbrand was obtained from the copepods in one instance, but as it was found no more it was considered that this might have been a contamination. A white facultative aerobic micrococcus was found among the copepods with Bacillus B. but no other organisms were isolated.

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[5] This net is one of particular design which was loaned for this work by the U. S. Bureau of Fisheries' Experiment Station, Woods Hole, Mass.

Hörhammer[6] demonstrated the presence of clumps of bacteria within the bodies of crustacea by staining them with neutral red or methylene blue. He worked principally on cyclops but mentioned having studied other copepods.

When the herring are held in the weirs they discard feces in appreciable quantities. The feces of those herring which have fed on copepods and schizopods float near the surface of the water in pink sections about 10 cm. long by about 5 cm. in diameter. One sample of this was collected with a little water from the southwestern part of Passamaquoddy Bay, not many miles from Wilson's Beach, where schizopods were found. A culture of B. walfischrauschbrand was isolated from the entire sample.

TABLE 1

TEMPERATURE CHANGES IN HERRING DURING TRANSIT

Method of Treating Herring in Transit	Amount of Feed Present	Time	Temperature in Degrees Centigrade	Remarks
Floated in brine containing 150 lbs. salt per hogshead of fish	Slight	6:30 a. m.	12	At weir
		7:00 a. m.	13	
		7:30 a. m.	15	
		8:00 a. m.	16	At factory
Dry-salted, 150 lbs. salt per hogshead. Herring 2½ feet deep in boat	47%	9:30 a. m.	11	
		10:36 a. m.	14	
		11:30 a. m.	18	
		12:30 p. m.	23½	
Dry-salted............	90%	5:56 a. m.	11	At weir (water same temperature)
		6:24 a. m.	12	
		9:23 a. m.	18½	
		10:36 a. m.	22	
		11:30 a. m.	24½	
		12:30 p. m.	27	
		4:20 p. m.	37½	At smoked herring factory

Determinations, which are recorded in Table 1, show that changes of temperature occur in masses of herring in the boats after being removed from the weirs, giving an increase sufficient to produce incubation and allow multiplication of bacteria. B. walfischrauschbrand or Bacillus B., if present in feed in the stomachs of the dead herring, probably began to multiply with the production of gas as soon as the temperature reached 25 C. This was undoubtedly the source of the gas bubbles mentioned above in the stomach and intestines. The gas thus produced by the decomposition of the feed may play an important mechanical part in the production of "belly-blown" sardines.

Herring which contain the copepods or shrimp when taken from the weirs decay very rapidly under ordinary conditions of transportation. The digestive tract sometimes becomes distended with gas, the body walls soften and fall away from the ribs as if they were a viscous liquid instead of flesh. Such herring are known as "belly blown.' Herring which were full of feed were however, transported for periods of 10 hours and held 12 hours longer in the laboratory when floated in water at 15 C. This method presented a rise of temperature sufficient to permit rapid bacterial multiplication. It also eliminated mechanical injuries to the fish.

"Feedy" fish were taken from the weirs in sterile jars and carried un-iced and unsalted to the laboratory. Samples were then taken aseptically from the stomachs and intestines and from different portions of the body. In all cases with a few exceptions, the flesh was free from bacteria. The contents of the digestive tract almost invariably contained B. walfischrauschbrand or Bacillus B. or both, but in no instance were they isolated from the flesh.

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[6] Arch. f. Hyg., 1910-1911, 73, p. 183.

Chemical tests[7] were made on masses of the feed removed from the stomachs aseptically when the above bacteria were isolated. Both ammonia and amins were found. Large quantities of schizopods and of copepods collected aseptically from the stomachs of the herring were incubated without added mediums or bacterial cultures. Chemical tests were then made from time to time of the decomposing substances. After 48 and 72 hours' incubations (Table 2), both kinds of feed contained large amounts of ammonia and smaller but appreciable quantities of amins. No chemical differences were detected between the decomposition of copepods and schizopods. Bacteriologic examinations made at the time of the removal of the chemical samples from the incubating masses showed B. walfischrauschbrand and Bacillus B. as the predominating organisms.

TABLE 2

AMMONIA AND AMINS IN SAMPLES OF "FEED" KEPT AT INCUBATOR TEMPERATURE (37.5 C.)

Sample No.	Description	When Taken			Incubated 24 Hours			Incubated 48 Hours			Incubated 72 Hours		
		Milligrams Volatile Nitrogen per 100 gm. as			Milligrams Volatile Nitrogen per 100 gm. as			Milligrams Volatile Nitrogen per 100 gm. as			Milligrams Volatile Nitrogen per 100 gm. as		
		Total	Ammonia	Amins	Total	Ammonia	Amins	Total	Ammonia	Amins	Total	Ammonia	Amins
1	Plankton from Woods Hole, Mass., kept at 30 C. Sample mostly composed of diatoms	2.82	...	...	....	....	....	....	....	....	4.14		
3	Plankton from St. Croix River off Campobello Island. Mostly composed of copepods	6.04	...	...	....	....	....	....	....	....	402.2		
7	Copepods from east and south of Campobello Island	3.94	...	...	272.4	206.2	66.2	468.2	375.1	93.1			
13	Copepods from shallow water east of Grand Manan	...	...	...	216.6	153.8	62.8						
18	Copepods from north end Grand Manan in very deep water	9.50	...	...	191.7	143.4	48.3	296.8	260.9	35.9			
5	Schizopods from Wilson's Beach Island, off Campobello	1.98	...	...	....	....	....	....	....	....	822.9	602.3	220.6
14	Schizopods from Wilson's Beach Island, off Campobello	3.95	...	...	220.4	88.2	132.2	787.6	573.7	213.9	1009.2	832.6	176.6
21	Feed taken from "belly-blown" fish at wharf, Eastport, Maine	37.7	17.1	20.6									
10	Fresh water herring from Taunton River, Taunton, Mass.	9.94	...	...	127.0	111.6	15.4	....	....	....	888.3	812.6	75.7

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[7] All chemical analyses were made by Mr. J. B. Wilson, assistant chemist, Animal Physiological Laboratory, Bureau of Chemistry, Washington, D. C.

Pure cultures of these bacteria were inoculated into the sterilized flesh of fish after enrichment with 0.2% dextrose and incubated at 37½ C. After varying intervals of incubation, chemical analyses were made, a bacteriologic check being made at the time of the removal of the chemical samples. The chemical results are recorded in Table 3. They show that both ammonia and amins were formed and that the amins were again less in amount than the ammonia. Positive tests for indol and skatol were also obtained.

TABLE 3

DETERMINATION OF AMMONIA AND AMINS IN PURE CULTURES OF BACILLUS WALFISCHRAUSCHBRAND AND BACILLUS B. GROWN IN THE LABORATORY

Laboratory No.	Kind of Bacteria	Time of Incubation, Days	Volatile Alkaline Material in Terms of					Nitrogen Determined by the Amino-Acid Method as Nitrogen per 1.0 gm. X_1, Mg.	Alkalinity as N/20 Acid per 1.0 gm., C c
			Milligrams Volatile Nitrogen per 100 gm. as			Percentage of Total as			
			Total	Ammonia	Amins	Ammonia	Amins		
30	Sterile	7	18.5	—	—	—	—	0.55	0.0
28	B. walfischrauschbrand	2	259.5	215.2	82.9	17.1	17.1	5.34	
28	B. walfischrauschbrand	4	—	—	—	—	—	5.65	
31	B. walfischrauschbrand	7	510.1	447.1	63.0	87.7	12.3	9.95	2.1
32	B. walfischrauschbrand	7	334.6	286.2	18.4	85.6	14.4	5.16	1.5
24	Bacillus B.	2	146.8	—	—	—	—	—	1.2
24	Bacillus B.	4	208.4	160.3	48.1	76.9	23.1	—	1.9
25	Bacillus B.	3	159.1	127.7	31.4	80.3	19.7	—	
29	Bacillus B.	2	145.8	132.6	13.2	81.0	9.0	2.52	
27	X_2 Bacillus B.	2	225.2	198.8	26.4	88.2	11.8	5.07	
27	Bacillus B.	3	—	—	—	—	—	5.59	

X_1 These determinations made by the later Van Slyke method and apparatus, J. Biol. Chem., 1913, **16**: 121.

X_2 A few micrococci in this culture.

In connection with feed in the stomach an interesting observation was made. Two silver hake were caught in herring weirs, one with 21 and the other 23 herring in its stomach. The stomachs were distended until they were approximately 4 inches in diameter at the widest portion. The section of the herrings which lay against the stomach lining had been markedly digested, but the remainder of the fish were perfectly firm and solid. In several of the herring there were copepods, but no gas either there or in their intestines. From two stomachs taken from herring lying in the central portion of one hake's stomach were found Bacillus B. and B. coli.

Among the references to the occurrence of B. coli in fish, Johnson[8] reports that G. C. Whipple found no B. coli in trout, perch and sunfish which were taken from an unpolluted water. Amyot's[9] conclusions, founded on the examination of 23 fish of 14 varieties caught near Port Stanley, Ontario, on Lake Erie, in August, 1901, agree with Professor Whipple's statement. Johnson, however, found B. coli to be present and to multiply in 15 varieties taken from the polluted waters of the Mississippi and Illinois Rivers. All of the fish which were found in this investigation to contain B. coli were taken from in or near sewage polluted water or water enriched with the trimmings of fish.

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[8] Jour. Infect. Dis., 1904, 1, p. 348.

[9] Tr. Am. Pub. Health Assn., 1901, 27, p. 400.

In an attempt to learn if the sterility of the digestive tract of other fish depends on the presence of feed, cod, rock fish, bass and Taunton (Mass.) River herring or alewives were examined. The alewives alone were obtained without feed present. Of 72 examined, 47 contained neither feed nor bacteria, 3 contained B. coli, and 22 contained bacteria but no gas-producing organisms.

From 2 cod, obtained at Eastport, Maine, one of which was freshly caught and one which had been out of water 2 hours, the following results were obtained. Both contained B. coli and B. walfischrauschbrand in the intestines and B. walfischrauschbrand in the gills. Both had partially digested feed in the stomach, of which the portions examined were sterile.

The curing of cod by drying[10] does not always kill the spores of B. enteritidis sporogenes. Cod are classed as bottom fish and are known to feed on refuse fish trimmings. Pollock, however, are often cured and sold as dried cod. These fish have often been seen feeding on schizopods in regions where the schizopods were found to contain B. walfischrauschbrand. It is therefore suggested advisable to examine commercial "dried cod" for B. walfischrauschbrand.

CONCLUSIONS

Swelling of processed cans of sardines is caused by an anaerobic spore-forming organism, which is probably identical with B. walfischrauschbrand of Nielsen.

B. walfischrauschbrand was isolated from factory dirt, from the gills of the herring, from feed (schizopods and copepods) found in the stomach and intestines of the fish where it had produced gas, from the thoracic and digestive portions of the schizopods and from the copepods as taken from the water.

Bacillus B. which was also found in the feed, especially in the copepods, pathogenic in peritoneal inoculation (guinea-pig) and produces gas in protein medium containing blood. It is killed at 65 C. in 20 minutes.

The fish while massed together in boats during transportation from the weirs to the cannery heat enough to permit rapid growth of B. walfischrauschbrand and Bacillus B. with the production of gas in the dorsal ends of the stomachs and intestines. Excess of such development tends to produce "belly-blown" fish, due to the active decomposition of the red-feed first and then of the fish.

The digestive tract of the herring was usually found sterile when no feed was present. The flesh was practically free from bacteria but organisms were present in the gills.

Removal of heads, gills, and viscera from such fish would reduce the danger of spoilage as due to these organisms. This danger is partially reduced by holding the fish in pounds until free from feed.

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[10] Stewart, C. Balfour: Thompson-Yates Laboratories, Report No. 3, 1901, p. 32.

B. walfischrauschbrand and Bacillus B. both survived frying in oil when the layer of fish was deep enough for 3 fish to overlap. They appear to be killed by heat to 240 F. for 3 minutes; if no fish are allowed to overlap.

B. walfischrauschbrand in the sealed cans will survive 1½ hours in a boiling tank, if a portion of the can is exposed, thus allowing a temperature even 1 or 2 degrees under the boiling point. To obtain efficiency all cans must remain entirely submerged and the water must boil freely.

B. coli and in some cases B. welchii were abundant in the water used in some canneries. For reasons of decency, as well as to reduce the loss of canned goods, the water used should be obtained far enough away from sewers to be free from sewage organisms.

DESCRIPTION OF ORGANISM

Bacillus Identified as B. Walfischrauschbrand

Morphology: Rod-shaped, gram-positive, occurs singly. Spores always present.

Size: 3.8-5 mikrons long by 0.8 mikrons wide.

Motility: Absent.

Spore Formation: A round spore located near one end of cell giving tennis-racket-shaped organism closely resembling the bacillus of symptomatic anthrax. Size of cell at spore end 1.8 mikrons.

Resistance: Resists heating in steam autoclave 10 minutes, killed by 15 minutes' heating. Culture in dextrose agar resisted 5 lbs. pressure for 5 minutes, killed at end of 8 minutes.

Relation to Temperature: Growth rapid at 37½ C. with production of spores. Slow growth at 25 C.

Reation to Air: Obligate anaerobe. Grows in agar shake tubes to within 3 cm. of surface.

Color: White.

Agar Containing: 1% of dextrose: Small white pine colonies in depth. Abundant gas at end of 18 hours' incubation at 37½ C. Spores. Odor foul.

Agar Streak: White, moist, slimy round colonies.

Milk: Coagulated.

Nutrient Broth: Slightly turbid, growth at bottom, foul odor.

Lactose Agar: Gas, acid to litmus.

Bacillus B.*

Morphology: Short, gram-positive rod.

Motility: Absent.

Spores: Not demonstrated.

Resistance: Thermal death point 65 C. for 20 minutes.

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* A large portion of this identification was prepared by W. G. Smillie, Harvard Medical School, Boston, Mass.

Cultivation: Grows best at 37½ C. in mediums containing 1 or 2 drops sterile horse blood.

Relation to Air: Obligate anaerobe, growing in agar tubes to within 2 cm. of surface.

Thermal Death Point: 65 C. for 20 minutes.

Blood Plates: 1 c c horse blood to 10 c c meat infusion agar. Forty-eight hours' growth. Small disk-shaped colony, margins regular. Around each colony is a zone of hemolysis. From 1-1.5 mm. in diameter.

Nutrient Agar: Grows only in depth of mediums within 2 cm. of surface. Abundant gas.

Dextrose: Abundant gas, foul odor.

Indol: Negative.

Animal Inoculation: 1 c c of a 24-hour bouillon culture to which 2 drops of horse blood were added was used to inoculate a guinea-pig intraperitoneally. Death in 18 hours. The animal was allowed to lie at room temperature (65 F.) for 18 hours for gas formation.

There were about 5 c c of hemolyzed discolored fluid in the abdomen; abdominal cavity was moderately distended with gas, not in the intestines alone, but free in the peritoneal cavity. Smear from the peritoneum shows large numbers of rods, which take the stain very readily.

Heart blood culture is positive for the gas forming rod.

There was used 0.5 c c of a 24-hour dextrose bouillon plus 2 drops of horse blood to inoculate a mouse intraperitoneally. Death ensued in 48 hours. The abdomen was not markedly distended. The animal was allowed to stay at the room temperature for 18 hours.

The abdomen was markedly distended Gas is free in the peritoneal cavity and there is also about 0.5 c c of hemoyzed blood, about 1 c c in pleural cavity. Smear from peritoneal and pleural cavity shows large numbers of short bacilli deeply staining.

Heart blood culture is positive for a gas-forming bacillus. Filtered 48-hour bouillon culture through W. Berkefeld filter.

Injected mouse, 0.5 c c, intraperitoneally.

Injected guinea-pig, 1 c c, intraperitoneally.

Mouse died in 12 hours. No fluid in peritoneum.

Necropsy negative.

Guinea-pig was very ill but survived the inoculation.

NOTE. — Since the completion of this work a reference has been found wherein the author states that the intestinal tract of certain fishes is free from bacteria when no food is present. Gillepsie, A. L., Fishing Board of Scotland, Report, 1898, p. 23.